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TITLE: Blood-Based Exosomal A-Synuclein Aggregates as a Quantifiable Biomarker of Parkinson's Disease

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CONTRACTING ORGANIZATION: VA Medical Center, Iowa City

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14. ABSTRACT

This study (W81XWH2010811/PD190043P1) aims to fulfill Objective 3 of the overall project titled as "Blood-Based Exosomal α-Synuclein Aggregates as a Quantifiable Parkinson's Disease Biomarker". Aims of this study are to 1) cross-validate the misfolded α-Synuclein in red blood cell (RBC)-derived extracellular vesicles (EVs) as a biomarker of PD in a cohort US veterans, and 2) investigate the misfolded αSyn in RBC-derived EVs as a potential biomarker of prodromal PD in a cohort of US Vietnam veterans exposed to Agent Orange but who have not been diagnosed with PD. Studies to fulfill Objectives 1 and 2 (developing the blood test and testing in animal models and humans) are conducted by our collaborators at Iowa State University and University of Washington. The study has been launched at the Iowa City VA Medical Center and is behind recruitment target due to the COVID-19 pandemic, but we are expediting enrollment as circumstances allow.

15. SUBJECT TERMS

NONE LISTED

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1. Introduction

This study (W81XWH2010811/PD190043P1) aims to fulfill Objective 3 of the overall project titled as "Blood-Based Exosomal α-Synuclein Aggregates as a Quantifiable Parkinson's Disease Biomarker". Aims of this study are to 1) cross-validate the misfolded α-Synuclein in red blood cell (RBC)-derived extracellular vesicles (EVs) as a biomarker of PD in a cohort US veterans, and 2) investigate the misfolded αSyn in RBC-derived EVs as a potential biomarker of prodromal PD in a cohort of US Vietnam veterans exposed to Agent Orange but who have not been diagnosed with PD. Studies to fulfill Objectives 1 and 2 (developing the blood test and testing in animal models and humans) are conducted by our collaborators at Iowa State University and University of Washington.

2. Keywords

Parkinson's disease, prodromal PD, α-Synuclein, synucleinopathy, RT-QuIC (real-time quaking-induced conversion) assays, neurotoxicity, Agent Orange, biomarker

3. Accomplishments

a. What were the major goals of the project?

i. *List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project identify these dates and show actual completion dates or the percentage of completion.*

Major Tasks: Months 1-36

1) Cross-validate the misfolded α-Syn in red blood cell (RBC)-derived extracellular vesicles (EVs) as a biomarker of PD in a cohort US veterans from the Iowa City VA Healthcare System comparing 60 PD patients with manifest PD against 60 healthy controls (HC) without PD.

We will conduct clinical assessments measuring motor severity (MDS-UPDRS) and cognitive function (MoCA), as well other clinical features affected by PD. We will draw blood and ship the samples to Iowa State University for RT-QuIC (real-time quaking-induced conversion) assays and to University of Washington for MSD ECL (Meso Scale Discovery immunoassay using electrochemiluminescence) analyses of RBC-EV αSyn_{agg}. RT-QuIC indices for misfolded protein aggregation are PAR (protein aggregation rate) and SD_{50} (median seeding index). The MSD ECL reveals levels of oligomerization by measuring phosphorylated (pS129)-αSyn concentrations.

We will compare the measures of RBC-EV α-Synuclein oligomerization and aggregation (pS129 α-Syn concentrations, PAR, and SD_{50}) between the manifest PD and control groups to see if these measures differentiate PD from controls. Within the PD group, we will correlate pS129 α-Syn concentrations, PAR, and SD_{50} with the measures of motor function (MDS-UPDRS) and cognition (MoCA) to determine if α-Synuclein oligomerization and aggregation levels in RBC-Ev are associated with the severity of PD.

2) Investigate the misfolded αSyn in RBC-derived EVs as a potential biomarker of prodromal PD in a cohort of 120 US Vietnam veterans exposed to Agent Orange but who have not been diagnosed with PD.

The prodromal PD status of AO exposed patients without a diagnosis of manifest PD will be determined using MDS research criteria (Heinzel S, Berg D, Gasser T, et al. Update of the MDS research criteria for prodromal Parkinson's disease. Mov Disord 2019;34: 1464-1470). AO-exposed participants will be considered having prodromal PD if their total likelihood ratio (LR) exceeds the 80% probability threshold based on age group.

We will compare the measures of RBC-EV α-Synuclein oligomerization and aggregation (pS129 α-Syn concentrations, PAR, and SD_{50}) between those who meet criteria for prodromal PD and who do not to see if these measures predict presence of prodromal PD in veterans exposed to AO and are at risk for PD. Within the AO exposed group, we will correlate pS129 α-Syn concentrations, PAR, and SD_{50} with the total LR to determine if α-Synuclein oligomerization and aggregation levels in RBC-Ev are associated with probability of prodromal PD.

b. **What was accomplished under these goals?**

    i. We enrolled 28 PD, 25 HC, and 2 AO participants.

c. **What opportunities for training and professional development has the project provided?**

    i. Nothing to Report.

d. **How were the results disseminated to communities of interest?**

    i. Nothing to Report.

e. **What do you plan to do during the next reporting period to accomplish the goals?**

    i. We will expedite enrollment to catch up with target enrollment.

4. Impact

a. **What was the impact on the development of the principal discipline(s) of the project?**

    i. Nothing to Report.

b. **What was the impact on other disciplines?**

    i. Nothing to Report.

c. **What was the impact on technology transfer?**

    i. Nothing to Report.

    ii. Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

        1. adoption of new practices: If successful, the project may lead to adoption of a blood biomarker test to diagnose and monitor Parkinson's disease in manifest and prodromal stages. This would likely improve diagnostic accuracy of PD and enable neuroprotective clinical trials in earlier phases of PD.

d. **What was the impact on society beyond science and technology?**

    i. "Nothing to Report."

    ii. Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

        1. *improving public knowledge, attitudes, skills, and abilities.* – If this project is successful, awareness of PD may rise due to ease of diagnosis. People with

symptoms of PD may seek medical attention earlier knowing that diagnosis is a relatively simple process.

2. *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or* - If this project is successful, PD may start to be diagnosed in the prodromal stage with non-motor symptoms only before emergence of motor manifestations. This can change diagnostic and treatment approach to PD. Insurance coverages may be affected. Preventive strategies such as exercise may gain more steam.

3. *improving social, economic, civic, or environmental conditions.* If this project is successful, early diagnosis and more effective treatments may increase potential for cure of PD, decreasing its financial burden on the society and caregivers.

5. Changes/Problems

a. **Changes in approach and reasons for change**

   i. No changes in approach

b. **Actual or anticipated problems or delays and actions or plans to resolve them**

   i. *Describe problems or delays encountered during the reporting period and actions or plans to resolve them.*

   Due to the COVID-19 pandemic we have not met our recruitment target. Our target population is of older age, typically in their 60s to 80s, which is at higher risk of poor outcomes and death from COVID-19. The risk of death from COVID-19 in older age gets further accentuated in patients with Parkinson's disease (Zhang et al., Coronavirus Disease 2019 Case Fatality and Parkinson's Disease. Mov Disord 2020;35:1914-1915).

   Due to the reasons above, we kept the study on administrative hold for several months after funding (until 2/10/2021) to avoid any unnecessary expenses. Furthermore, although the administrative hold on the study was lifted on 2/10/2021, the Iowa City VA Medical Center has operated under strict rules until late Spring/early Summer of 2021 to mitigate COVID-19 pandemic which included social distancing with limitation of in-person outpatient visits to the hospital in favor of virtual visits unless in-person encounters were medically necessary.. Therefore, we could not start recruiting in February 2021. We started to enroll and test participants in mid-Summer 2021 and have optimized the workflow for recruitment, testing, and shipment of samples to collaborating labs. Despite the delta and omicron waves, we continued to recruit and have been able to enroll 55 subjects so far (43 in FY22). With hopeful easing of the pandemic, we plan to expedite our recruitment to catch up with recruitment goals.

c. **Changes that had a significant impact on expenditures**

   i. Due to COVID-19 pandemic related lack of clinical research activity in the initial months there was less personnel expense.

d. **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

   i. N/A

e. **Significant changes in use or care of human subjects:** None

f. **Significant changes in use or care of vertebrate animals.** N/A

g. **Significant changes in use of biohazards and/or select agents:** N/A

6. Products

a. **Publications, conference papers, and presentations**
Report only the major publication(s) resulting from the work under this award.

i. **Journal publications.** *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

1. Nothing to report yet

ii. **Books or other non-periodical, one-time publications.** *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

1. Nothing to report yet

iii. **Other publications, conference papers, and presentations.** *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

1. Nothing to report yet

b. **Website(s) or other Internet site(s)**
Nothing to report.

c. **Technologies or techniques**
Our part of the study is not to develop technology, but validate technology developed by our collaborators at the Iowa State University and University of Washington.

d. **Inventions, patent applications, and/or licenses**
Nothing to report

e. **Other Products**
Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

i. Nothing to report

7. Participants & Other Collaborating Organizations

a. **What individuals have worked on the project?**

i. *Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."*

a.

Name:	Ergun Y. Uc, MD
Project Role:	Partnering PI
Researcher Identifier (e.g. ORCID ID):	ORCID ID 0000-0003-4430-6960
Nearest person month worked:	*2*
Contribution to Project:	Dr. Uc has hired a research nurse and continued meetings with the other teams of the Lead PI Dr. Kanthasamy and Dr. Tessandra Stewart to streamline research procedures. Dr. Uc has laid the groundwork for subject recruitment. After fully training the research personnel and establishing logistical support, he started recruiting patients.
Funding Support:	Besides this DoD grant, Dr. Uc has funding support from the NINDS and VA and the Parkinson's Foundation for other projects.

Name:	Michelle Lenz, RN BSN
Project Role:	Research nurse and coordinator
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	*4*
Contribution to Project:	Ms. Lenz tests participants, does data entry, coordinates shipments of blood to the labs of other collaborators
Funding Support:	No other funding

b. **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

i. No

c. **What other organizations were involved as partners?**

i. *Describe partner organizations - academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) - that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed. Provide the following information for each partnership:*

1. **Organization Name:** Iowa State University/University of Georgia in Athens

    a. **Location of Organization:**

    b. **Partner's contribution to the projec**t

        i. **Financial support -** none

        ii. **In-kind support** – Coordinates activities between collaborating centers, organizes meetings between centers; provides material for sample collection

        iii. **Facilities** -none

        iv. **Collaboration** – Dr. Kanthasamy is the initiating PI under the contract award number W81XWH-20-1-0781 and grant proposal number PD190043. Dr. Kanthasamy moved to University of Georgia during this annual report year, but has still connections with the Iowa State University. Dr. Kanthasamy's lab assists with analyses of samples we sent. Overall statistical analyses will be performed by Dr. Kanthasamy's team.

        v. **Personnel exchanges** -None

        vi. **Other.**- none

2. **Organization Name:** University of Washington

    a. **Location of Organization:**

    b. **Partner's contribution to the projec**t

        i. **Financial support -** none

        ii. **In-kind support** – guides with sample collection details

        iii. **Facilities** -none

        iv. **Collaboration** - Dr. Stewart in another partnering PI of Dr. Kanthasamy under the contract award number W81XWH-20-1-0784 and grant proposal number PD190043P2. Dr. Stewart's lab analyzes blood samples we send.

        v. **Personnel exchanges** -None

        vi. **Other.**- none

3. **Organization Name:** VA Medical Center of Iowa City, part of Iowa City VA Health Care System (This is actually the center where the study is conducted)

    a. **Location of Organization:**

    b. **Partner's contribution to the projec**t

        i. **Financial support -** none

        ii. **In-kind support** – Phlebotomy and initial sample processing/storage at the Iowa City VA Lab; participants are recruited from Iowa City VA

        iii. **Facilities** - The Iowa City VA Research Foundation, Dr. Uc's office and lab are in the Iowa City VA Medical Center. Dr. Uc is recruiting participants from the clinics of Iowa City VA Medical Center.

        iv. **Collaboration** – The Iowa City VA Medical Center is Dr. Uc's location of clinical practice and research for this study.

        v. **Personnel exchanges** -None

        vi. **Other.**- none

8. Special Reporting Requirements

a. **COLLABORATIVE AWARDS:** *For collaborative awards, independent reports are required from **BOTH** the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to https://ebrap.org for each unique award.*

   Dr. Kanthasamy, the initiating PI under the contract award number W81XWH-20-1-0781 and grant proposal number PD190043, will submit their own report.

   Dr. Stewart, another partnering PI of Dr. Kanthasamy under the contract award number W81XWH-20-1-0784 and grant proposal number PD190043P2, will submit their own report.

b. **QUAD CHARTS:** *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

   Submitted separately.

9. Appendices

None